REMARKS

The May 3, 3002 Official Action and the references cited therein have been carefully considered. In view of the amendments presented herewith and the following remarks, favorable reconsideration and allowance of this application are respectfully requested.

At the outset, it is noted that a shortened statutory response period of three (3) months was set in the May 3, 2002 Official Action. The initial due date for response, therefore, was August 3, 2002. A petition for a three (3) month extension of the response period is presented with this response, which is being filed within the three (3) month extension period, as November 3, 2002 fell on a Sunday.

As another preliminary matter, it is noted that the requirement for restriction set forth in the February 4, 2002 Official Action (Paper No. 7) has been repeated and made final. The Examiner acknowledges applicants' election of the subject matter of claims 1-7, 13 and 16 (Group I), and states that new claims 17-30 and 38-40 are drawn to the elected subject matter of Group I and that those claims will be examined in this application. Claim 16 should be examined herein, as well, in that it is in the group of claims which applicants elected and it remains pending. The Examiner is incorrect in stating, under the heading "Application Status" at page 2 of the May 3, 2002 Official Action, that applicants canceled all previously pending claims.

Applicants once again reserve the right to file one or more continuing applications, as provided under 35 U.S.C. §121, on the subject matter of the claims that are withdrawn from consideration by the Examiner in this application.

Certain informalities in the drawings submitted with this application and deficiencies constituting incomplete compliance with the sequence listing requirements of 37 C.F.R. §1.821 *et seq.* are noted in paragraphs 5 and 6 of the May 3, 2002 Official Action. In accordance with the present amendments, applicants have inserted appropriate sequence identifiers at the specification pages indicated in paragraph 6(d) of the May 3, 2002 Official Action. As for the required drawing corrections, it is respectfully requested that this requirement be held in abeyance, pending an indication of allowable subject matter. At such time as the drawings are corrected, sequence identifiers for Sequence ID Nos. 1-25, 54 and 55 will be added to Figures 2, 4 and 7 of the drawings, as appropriate.

In the May 3, 2002 Official Action, the specification was objected to in several respects, including an objection to the title for inadequately describing the claimed subject matter; an objection to the Abstract for not defining the abbreviation "CLF"; an objection to the Brief Description of the Drawings, for lack of a section heading; an objection to the description of the drawings, *per se*, for lack of an appropriate reference to the multi-sheet drawings of Figures 2 and 4; and an objection based on the stray markings appearing in the chemical structures shown

at pages 35, 45, 51 and 56 of the present specification.

A number of objections were also raised with respect to the claims, based on the informalities noted in paragraph 11 of the May 3, 2002 Official Action. Claim 20 is also deemed objectionable by the Examiner as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. According to the Examiner, the limitation of having an arginine in the active site of the AT domain does not further limit the subject matter of the parent claim for two reasons, namely, the active site is undefined and every AT domain has an arginine somewhere; and an arginine about position 110 is virtually ubiquitous in known AT domains.

In addition, claims 26 and 38 are deemed objectionable under 35 U.S.C. §132 as allegedly introducing new matter into the disclosure.

Turning to the grounds of rejection set forth in the May 3, 2002 Official Action, claims 17-30 stand rejected as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. In this connection, the Examiner notes, in paragraphs 13-18 of the May 3, 2002 Official Action, a number of claim terms that appear in certain claims and are characterized as unclear and a number of abbreviations, the definitions of which are not made plain in the Examiner's view.

In addition to the above-noted objections, claims 26 and 38 are separately rejected under 35 U.S.C. §112, first

paragraph, as the specification allegedly lacks a written description of the subject matter of those claims, such as would reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Claims 17-30 and 38-40 are also rejected under 35 U.S.C. §112, first paragraph, also for lack of written description on the authority of University of California v. Eli Lilly & Co., 43 U.S.P.Q.2d 1398 (Fed. Cir. 1997); cert. denied, 523 U.S. 1089 (1998). The Examiner concedes that numerous species within the claimed genus are exemplified in the specification, but nevertheless contends that the specification is deficient with respect to the common structural and functional characteristics of the claimed molecules. The Examiner further asserts that without such structural/functional descriptions of the genus of claimed molecules, the specification lacks an adequate written description thereof.

Claims 17-30 and 38-40 are further rejected under 35 U.S.C. §112, first paragraph as allegedly based on a specification that provides inadequate enablement for the full scope of the claims. In this connection, the Examiner asserts that the specification does not enable one skilled in the art to make the invention commensurate in scope with these claims, and that to enable the claimed genus would require undue experimentation.

Claims 17, 18, 20-22, 24, 28, 29 and 30 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Kuhstoss et al., Gene 183:231-36 (1996).

Claims 19, 23, 25 and 38-40 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over the combined disclosures of U.S. Patent No. 5,712,146 to Khosla et al. (Khosla I) and Khosla Chem. Revs., 97:2577-90 (1997) (Khosla II). According to the Examiner, it would have been obvious to one of ordinary skill in the art to use the teachings of Khosla I to produced the claimed invention because the cited reference purportedly describes all possible combinations of genes, modules, domains and portions thereof. As for motivation for the proposed combination of references, the Examiner asserts that one would have been motivated to produce PKS enzymes because of the great therapeutic potential of novel polyketides that can be easily produced, in combinatorial fashion, using the system of mixing and matching described by Khosla I. The Examiner further maintains that one would have had a reasonable expectation of success that such combination of genes, modules, domains and portion thereof would render functional polyketides due to the extensive similarities among modular and PKS enzymes, as purportedly suggested by Khosla II.

The foregoing objections and rejections constitute all of the grounds set forth in the May 3, 2002 Official Action for refusing the present application.

In accordance with the foregoing amendments, a new title has been presented, in accordance with the Examiner's suggestion.

The Abstract has been amended to indicate what is meant by "CLF". As is well known, "CLF" is a term of art. Its meaning is discussed in the present specification at page 5, line 19, through page 6, line 22. As there explained, "CLF" stands for "chain length factor". However, because of a mistaken belief about its function, it has also been termed "KS beta". Accordingly, both of these designations are indicated in the amended Abstract.

The specification has been amended by inserting an appropriate section heading for the Brief Description of the Drawings; and references to the multiple sheet drawings of Figures 2 and 4 have been added.

Regarding the chemical structures appearing at pages 35, 45, 51 and 56 of the specification, applicants wish to point out that the faint diagonal and the other stray marks at the ends thereof are unintended artifacts of word processing. Here again, it is respectfully requested that correction of these chemical structures be held in abeyance pending an indication of allowable subject matter.

The present claim amendments include amendments to claim 17-20 and 28-30 which are believed to overcome the objections noted in paragraph 11 of the May 3, 2002 Official Action. Similar amendments have been made to the form of claim

16.

In addition, the proviso of claim 17 has been amended to include that the synthase is not composed of the loading module of the tylosin polyketide synthase coupled to the spiramycin polyketide synthase, minus its natural loading module. Support for this addition to the proviso of claim 17 is provided by the discussion of the paper by Kuhstoss et al. appearing at page 12, line 6 through page 13, line 8 of the present specification. This amendment to claim 17 is proper when considered in light of In re Johnson, 194 U.S.P.Q. 187 (C.C.P.A. 1977) and In re Driscoll, 195 U.S.P.Q. 434 (C.C.P.A. 1977). In both of these cases, applicant was permitted narrowing amendments to avoid having claims read on subject matter that the applicant was not entitled to claim.

Given that the Kuhstoss et al. paper evidences no appreciation whatsoever about the function of the KSq domain, the experiment described by Kuhstoss et al., although successful in the instance described, teaches nothing about the generality of applicants' invention. Kuhstoss et al. actually teach away from the true role of the KSq at page 13, lines 2-8.

The present amendments are also believed to overcome the 35 U.S.C. §112, second paragraph rejections set forth in paragraphs 13-18 of the May 3, 2002 Official Action.

Claims 22, 29 and 39 have been amended to include recitations further characterizing "KSq" and claim 22 has been amended to further characterize "ATq". Support for these

amendments is provided in the present specification at page 11, line 18-24 and at page 17, lines 15-18.

Claim 26 has been amended to recite that the AT domain corresponds to the acyltransferase of module 6 of the niddamycin polyketide synthase. Support for this amendment is provided at page 27, lines 3-12 of the present specification.

New claims 41-43 are also presented with this amendment.

New claim 41 is based essentially on original claim 15, but is directed to a PKS, rather than a process. It also finds support in the passage from page 23, line 16 through page 24, line 19. New claim 42 depends from claim 41, and is based on page 24, lines 8 through 12 of the specification.

New claim 43 starts out like claim 17, but contains a different definition of the "loading module". This is defined in a manner similar to the disclosures at page 19, line 9. The AT domain is defined simply as "an acyltransferase domain which is adapted to load an optionally substituted malonyl". This differs in scope from the "ATq" in the formula on page 19. This difference is believed to be warranted by the passage bridging pages 22 and 23 of the specification.

The first domain is labeled "Dec" to encompass both Ksq and CLF. It is defined in terms based on page 18, lines 12 to 13 of the specification. Claim 43 further recites that at least one of the domains is heterologous or is engineered. This recitation is based on page 22, line 23 to page 23, line 2 of the

specification. Since this claim requires there to have been genetic engineering and/or hybrid formation within the loading domain, it is believed to be clearly distinguished from the disclosure of the Kuhstoss et al. paper.

No new matter has been introduced into this application by reason of any of the amendments presented herewith. For the most part, the effect of the foregoing amendments is merely to make express that which was implicit in the application as originally presented.

In view of the claim amendments presented herewith, any indefiniteness or lack of clarity that may have been engendered by the prior wording of 17-30 and 38-40 has now been eliminated. Thus, the only matters remaining to be addressed are the objection to claim 20 as being of improper independent form and the various grounds of rejection set forth in paragraphs 19-23 of the May 3, 2002 Official Action. Applicants respectfully submit that these last-mentioned grounds of rejection and objection either lack merit or cannot be maintained in view of the present amendments, or both. These rejections and objection are, therefore, respectfully traversed.

A. Claim 20 is a Proper Dependent Claim, As it Further Limits the Subject Matter of Claim 17

It appears from the very statement of this objection that the Examiner misapprehends the import of the arginine residue at the active site of the acyltransferase in loading domains. Although arginine residues at this position are

ubiquitous in extender acyltransferases, such is not the case in loading acyltransferases. Loading acyltransferases which do not have the KSq domain do not have an arginine at this position, whereas those which do have a KSq domain do have the active site arginine. Claim 20, therefore, does further limit claim 17 in a material way. This is fully discussed at page 17 lines 5-15 of the present specification.

Inasmuch as claim 20 further limits claim 17, for the reasons given above, the objection to claim 20 as an improper independent claim should be withdrawn.

B. Claims 17-30 and 38-40 Fully Comply with the Written Description Requirement of 35 U.S.C. §112, First Paragraph

The relevant inquiry in determining compliance with the written description requirement of 35 U.S.C. §112, first paragraph, is whether the originally filed specification reasonably conveys to a person having ordinary skill in the art that applicant had possession of the claimed subject matter. In re Kaslow, 217 U.S.P.Q. 1089 (Fed. Cir. 1983).

Furthermore, the Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in applicants' specification disclosure a description of the invention defined by the claims. Ex parte Sorenson, 3 U.S.P.Q.2d 1462 (Bd. Pat. App. 1987).

The Examiner's reliance on University of California v. Eli Lilly & Co. supra, is clearly misplaced. The Federal Circuit

in that case stated, at 1406:

> A description of a genus of CDNA's may be achieved by means of a recitation of a representative number of CDNA's, defined by nucleotide sequence, falling with the scope of the genus.... This is analogous to enablement of a genus under §112, ¶1, by showing the enablement of a representative number of species within the genus. See Angstadt, 537 F.2d at 502-503, 190 U.S.P.Q. at 218 (deciding that applicants 'are not required to disclose every species encompassed by their claims even in an unpredictable art'....). [Emphasis added; footnote omitted.]

In the present case, applicants have satisfied the written description requirement of §112 by describing a representative number of the type I polyketide synthases within the generic definitions set out in claims 17, 38 and 39. Indeed, applicants believe that Figure 4 encompasses all of the specific examples known in the art at the time the present invention was made.

The Examiner acknowledges that these specific examples are numerous, but criticizes the specification for allegedly failing to identify common structural and functional characteristics of the polyketide synthases. This criticism is unfounded, however, as the common characteristics of KSq-containing loading modules are fully described in both structural and functional terms in the specification. Specifically, the KSq-containing loading module is fully described in structural terms, both in specific instances at page 11, line 6 through page 12, line 6 and page 15, line 24, through page 17, line 18; and more generally in other places within the text, for example, page

17, line 19 through page 19, line 10. Moreover, the KSq-containing loading module is fully described in functional terms both in specific instances at page 16, lines 4 through 21 and, more generally at page 17, line 24 through page 18, line 4.

For all of the foregoing reasons, it is respectfully submitted that in the present case the Examiner has failed to satisfy her burden of proof with respect to the lack of written description requirement rejection, as applied to the subject matter of claims 17-30 and 38-40. Accordingly, this ground of rejection is untenable and should be withdrawn.

As for the lack of written description/new matter rejection of claims 26 and 38, this rejection, likewise, cannot be maintained.

Claim 26, as originally presented, recited that the AT domain "is specific for loading with hydroxymethylmalonyl". This aspect of the invention is described at page 17, line 19 through page 18, line 2 of the specification, where it is stated that "the loading module is adapted to load a malonyl or substituted malonyl residue and then to effect decarboxylization of the loaded residue". At page 19, lines 8 and 9 of the specification, it is further stated that "usually the loading module will be of the form: PKq-ATq-Acp". The specification further discloses at page 27, lines 3-12 that novel polyketides are obtainable "by the action of a KSq domain on the enzyme-bound product of an AT of unusual specificity derived from an extension module of a natural type-I PKS. In particular...the AT of extension module 6 of

niddamycin PKS gene cluster preferentially incorporates a side chain of structure HOCH2-". In other words, this AT loads a substituted malonyl whose side chain is HOCH2-, i.e., it is hydroxymetholmalonyl as stated in claim 26. Therefore, the subject matter of claim 26 does not constitute new matter. The same is true of the amendment to claim 26 presented herewith, which is clearly supported by the above-quoted passages of applicants' specification.

Regarding the proviso of claim 38, support is provided by the discussion of the Kuhstoss et al. paper, appearing at page 12, line 6, through page 13, line 8 of the present specification.

In summary, neither claim 26 nor claim 38 can properly be rejected for lack of written description or on new matter grounds. These rejections should, therefore, be withdrawn.

C. Claims 17-30 and 38-40 Fully Comply with the Enablement Requirement of 35 U.S.C. §112, First Paragraph

A rejection under 35 U.S.C. §112, first paragraph, based on alleged inadequate enablement is proper only when the rejected claims are of such breadth as to read on subject matter as to which the subject matter is not enabling. In re Borkowski, 164 U.S.P.Q. 642 (C.C.P.A. 1970).

Moreover, it is settled law that whenever the adequacy of enablement provided by an applicants' specification is challenged, the PTO has the initially burden of giving reasons, supported by the record as a whole, why the specification is

considered inadequate. In re Armbuster, 185 U.S.P.Q. 152 (C.C.P.A. 1975). A properly supported showing that the disclosure entails undue experimentation is part of the PTO's initial burden under §112, first paragraph. In re Angstadt, 190 U.S.P.Q. 214 (C.C.P.A. 1976).

The present specification describes in considerable detail the structure and organisation of type I PKSs and their encoding genes, and genetic manipulation thereof. See, for example, page 2, line 15 through page 4, line 21. Furthermore Figure 1 illustrates the organisation of the modules and domains in the erythromycin PKS as a prototype for all type I PKSs, the genes encoding the PKS, and the relationship with the polyketide chain produced and lactonised by the PKS. Figure 3 shows the module and domain organisation of a further 3 type I PKSs. The references cited fairly comprehend and reflect the understanding of those skilled in the art with respect to structure and organisation of PKSs and their encoding genes - in particular, Cortes et al (1990), Donadio et al. (1991), MacNeil et al., (1992), Swan et al., (1994), Schwecke et al. (1995), Cortes et al (1995), Haydock et al. (1995), Hutchinson & Fujii (1995), PCT/GB97/01819, PCT/GB97/01810, EP 0791655A2, WO95/08548, Kuhstoss et al. (1996) and Kavakas et al. (1998). These references should be taken into account in assessing the scope of enablement provided by the present specification. Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986.

Given the descriptions in the text, the cited references and the working examples, anyone skilled in the art could replicate the invention in any type I PKS whose gene sequence was known, without undue experimentation.

In contrast to the clear teaching provided by applicants, the Examiner has failed to provide any evidence or reasoning, supported by the record or otherwise, challenging the correctness of applicants' disclosure in this regard, as is required by the above-cited case law. Nor has the Examiner identified any specific subject matter within the scope of applicants' claims for which the present specification is considered non-enabling. An unsupported reference to unpredicatability in the art is simply not adequate.

In the absence of adequate evidence or reasoning to support the Examiner's position, the rejection of claims 17-30 and 38-40 based on alleged inadequate enablement cannot be maintained.

D. Claims 17, 18, 20-22, 24, 28, 29 and 30, as Now Amended, are Patentable Over Kuhstoss et al.

The amendment to the proviso of claim 17, reciting that "the synthase is not composed of the loading module of the tylosin polyketide synthase coupled to the spiramycin polyketide synthase minus its natural loading module", clearly establishes the novelty of claims 17, 18, 20-22, 24, 28, 29 and 30.

The Examiner has acknowledged in paragraph 24 at page 15 of the May 3, 2002 Official Action that Marsden et al. cannot

be used as prior art against claim 17, among others, in view of the proviso recited in claim 17. Similar reasoning compels the conclusion that Kuhstoss et al. cannot be cited as evidence of lack of novelty with respect to the subject matter of claims 17, 18, 20-22, 24, 28, 29 and 30, in view of the amended proviso of claim 17.

Accordingly, the 35 U.S.C. §102(b) rejection of claims 17, 18, 20-22, 24, 28, 29 and 30 is untenable and should, therefore, be withdrawn.

E. The Combined Disclosures of Khosla I and Khosla II Fail to Render Obvious the Subject Matter of Claims 19, 23, 25 and 38-40

The disclosure of Khosla II patent is mainly concerned with type II systems. Its disclosure relevant to type I systems is scanty and very general in nature. The Examiner cites Column 19, lines 12-20. The cited passage refers to Fig. 9, which shows the structure of the DEBS type I PKS. It can be seen from this that Khosla et al. were unaware of the existence of loading modules. "MODULE 1" of Fig. 9 actually designates the loading module AND the first extension module. Thus plainly it is impossible to extract from this reference any teaching relevant to the present invention. Essentially the same diagram appears in Fig. 3 of Khosla II.

The Examiner states that column 9, lines 47-50 teach type I/type II hybrids "which would include a loading domain containing a CLF domain in a PKS enzyme". This passage is merely

part of the definitions section, explaining how the term "replacement PKS gene cluster" will be used. Even if this is taken as a disclosure of something containing a portion of type I and a portion of type II, it certainly does not disclose what the portions are or where they come from. There is certainly no necessity for them to include a CLF domain, as the Examiner alleges. The loading domain could come from a type I PKS, and would then be joined to its native first extension module, since neither Khosla I nor Khosla II suggest that it has any independent significance.

In summary, the cited Khosla I approach of using a combination of enzymes, modules, active sites or portions thereof derived from aromatic, modular or fungal PKS gene clusters is a random approach. At best, it simply suggests throwing the genes into the cellular bag and evaluating whether they produce any polyketide. Furthermore, neither Khosla I nor Khosla II evinces any recognition whatsoever of the role of the CLF, but in fact misidentified its function and, therefore, could not appreciate its utility in the context of this invention.

Applicants' claims by contrast, relate specifically to type I PKS systems, in which a particular type of loading module ("decarboxylating") is joined to a heterologous extension module. Unlike Khosla I, applicants' approach is a precise and tailored approach based on understanding of the enzyme function and methods of precisely engineering type I PKSs within the open

reading frame. This is not at all suggested by Khosla I or Khosla II.

In view of the above-noted deficiencies in the disclosure of Khosla I and Khosla II, it is clear that these references fail as evidence of unpatentability in this case, because they do not place applicants' invention in the possession of the public. It has long been held that when a prior art reference is relied on to show or suggest that an invention purportedly disclosed therein is unpatentable, such reference must place the invention in the possession of the public. In re Brown, 141 U.S.P.Q. 245 (C.C.P.A. 1964). An invention is not "possessed by the public" absent a known or obvious way to make it. In re Payne, 203 U.S.P.Q. 245 (C.C.P.A. 1979). The same principles are articulated in §2121.01 of the Manual of Patent Examining Procedure.

The approach disclosed by Khosla I and Khosla II does not produce any hybrid polyketides apart from pure type IIs, notwithstanding the Examiner's contention to the contrary. The combined disclosures of Khosla I and Khosla II certainly do not teach or suggest the type I polyketide synthases called for in applicants' claims 19, 23, 25 and 38-40.

An obviousness rejection of applicants' claims to type I polyketide synthases based on such nebulous disclosure as that provided by Khosla I and Khosla II is manifestly untenable. As noted by the Board of Appeals in Ex parte Saceman, 27 U.S.P.Q.2d 1472 (P.T.O. B.P.A.I. 1993), the legal conclusion of obviousness

must be supported by facts. When the legal conclusion is not supported by facts, it cannot stand. To the same effect is In re Burt, 148 U.S.P.Q. 548 (C.C.P.A. 1966), in which the Court observed that silence in a reference is not a proper substitute for adequate disclosure of facts from which a conclusion of obviousness may justifiably follow. In the present case, the Examiner' position regarding the alleged obviousness of applicants' type I polyketide synthases, as called for in applicants' claims 19, 23, 25 and 38-40, has no factual basis in the prior art of record and, therefore, cannot be maintained.

In view of the present amendments and the foregoing remarks, it is respectfully requested that the objections and rejections set forth in the May 3, 2002 official Action be withdrawn and that this application be passed to issue, as such action is earnestly solicited.

Respectfully submitted,

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Marked-Up Version of Replacement Paragraphs on Page 29, line 14 Through Page 31, Line 3

Brief Description of the Drawings

Some embodiments of the invention will now be described with reference to the accompanying drawings in which:

Fig 1 is a diagram showing the functioning of 6-deoxyetythronolide B synthase (DEBS), a modular PKS producing 6-deoxyerythronolide B (6-DEB) a precursor of erythromycin A.

[Fig 2 gives] Figs. 2A-2D give the amino acid sequence comparison of the KS domains and the CLF domains of representative Type II PKS gene clusters. The active site Cysteine (C) of the KS domains is arrowed in the Figure and aligns with the Glutamine (Q) or glutamic acid (E) of the CLF domains. The abbreviations used, and the relevant Genbank/EMBL accession numbers are: GRA: granaticin from *Streptomyces violaceoruber* (X63449); HIR: unknown polyketide from *Saccharopolyspora hirsuta* (M98258); ACT, actinorhodin from *Streptomyces coelicolor* (X63449); CIN: unknown polyketide from *Streptomyces cinnamonensis* (Z11511); VNZ: jadomycin from *Streptomyces venezuelae* (L33245); NOG: anthracyclines from *Streptomyces nogalater* (Z48262); TCM: tetracenomycin from *S. glaucescens* (M80674); DAU: daunomycin from *Streptomyces sp.* C5 (L34880); PEU, doxorubicin from *Streptomyces peucetius* (L35560); WHI: WhiE spore pigment from *Streptomyces coelicolor* (X55942).

Fig 3 shows the gene organisation of the PKSs for three 16-membered ring macrolides, tylosin, spiramycin and-niddamycin.

[Fig 4 shows] Figs. 4A-4C show the amino acid sequence alignment of KSq-ATq loading didomains of the PKSs for niddamycin, platenolide(spiramycin), monensin, oleandomycin and tylosin. The sequences for the monensin and oleandomycin loading didomains have not been previously disclosed.

Fig. 5 The enyzmatic steps that convert 6-deoxyerythronolide B into erythromycin A in *Saccharopolyspora erythraea*.

Fig. 6 is a diagram showing the construction of plasmid pJLK117.

Fig. 7 shows the structures of two oligonucleotides.

Marked-Up Version of Replacement Paragraphs

(Paragraph at page 32, line 13 through page 33, line 10.) The following synthetic oligonucleotides: 5' - CCATATGGCCGCATCCGCGTCAGCGT- 3' (SEQ ID No. 28) and 5' - GGCTAGCGGGTCCTCGTCCGTGCCGAGGTCA- 3' (SEQ ID No. 29) are used to amplify the DNA encoding the putative monensin-producing loading module using a cosmid that contains the 5' end of the putative monensin-producing PKS genes from *S. cinnamonensis* or chromosomal DNA of S.*cinnamonensis* as template. The PCR product of 3.3 kbp is purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which has been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL40. Plasmid pPFL40 was identified by restriction pattern and sequence analysis.

(Paragraph at page 33, line 11 through page 33, line 2.) Plasmid pHD30His is a derivative of pNEWAVETE (PCT/GB97/01810) which contains the avermectin loading module, erythromycin extension modules 1 and 2 and the ery thioesterase domain. Plasmid pNEWAVETE was cut with is EcoRI and HinDIII and a synthetic oligonucleotide linker was inserted that encodes the addition of a C-terminal polyhistidine tail to the polypeptide. The following oligonucleotides:

5' -AATTCACATCACCATCACCATCACTAGTAGGAGGTCTGGCCATCTAGA- 3' (SEQ ID No. 30) and

5' -AGCTTCTAGATGGCCAGACCTCCTACTAGTGATGGTGATGGTGATGTG- 3' (SEQ ID No. 31) were annealed together and the duplex was ligated to EcoRI-and HinDIII-cut pNEWAVETE. The resulting plasmid was cut with NdeI and XbaI and ligated into plasmid pCJR24 that had been previously cut with same two enzymes, to produce plasmid pND30His.

(Paragraph at page 43, line 24 through page 44, line 7.) The following synthetic oligonucleotides: 5' - CCATATGACCTCGAACACCGCTGCACAGAA- 3' (SEQ ID No. 54) and 5' - GGCTAGCGGCTCCTGGGCTTCGAAGCTCTTCT- 3' (SEQ ID No. 55) were used to amplify the DNA encoding the tylosin-producing loading module using either cos6T (a cosmid that contains the tylosin-producing PKS genes from *S. fradiae*) or chromosoml DNA from *S. fradiae* as template. The PCR product of 3.3 kbp was purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid PpPFL39. Plasmid pPFL39 was identified by restriction and sequence analysis.

(Paragraph at page 47, lines 13-19.) A 411 bp DNA segment of the *eryA*I gene from *S.erythraea* extending from nucleotide 1279 to nucleotide is 1690 (Donadio, S. *et al.*,

Science (1991) 2523:675-679) was amplified by PCR using the following synthetic oligonucleotide primers: 5' -TGGACCGCCGCCAATTGCCTAGGCGGGCCGAACCCGGCT- 3' (SEQ ID No. 32) and 5' -CCTGCAGGCCATCGCGACGACCGCGACCGGTTCGCC- 3' (SEQ ID No. 33).

(Paragraph at page 49, line 13 through page 50, line 5.) A DNA segment encoding the KSq domain from the *ole*AI gene of *S. antibioticus* extending from nucleotide 1671 to nucleotide 3385 was amplified by PCR using the following synthetic oligonucleotide primers: 5' -CCACATATGCATGTCCCCGGCGAGGAA- 3' (SEQ ID No. 34) and 5' -CCCTGTCCGGAGAAGAGGAAGGCGAGGCCG- 3' (SEQ ID No. 35) and chromosomal DNA from Streptomyces antibioticus as a template. The PCR product was treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL31. The new *Nde* I site bordering the insert is adjacent to the *Eco* RI site of the pUC18 polylinker while the new *Bsp* EI site borders the *Hin* dIII site of the linker region. Plasmid pPFL31 was identified by restriction and sequence analysis.

(Paragraph at page 53, line 18 through page 54, line 7.) The following synthetic oligonucleotides: 5' -CCATATGTCTGGAGAACTCGCGATTTCCCGCAGT- 3' (SEQ ID No. 36) and 5' -

GGCTAGCGGGTCGTCGTCGTCCCGGCTG- 3' (SEQ ID No. 37) were used to amplify the DNA encoding the spiramycin producing loading module using chromosomal DNA from the spiramycin producer *S. ambofaciens* prepared according to the method described by Hopwood *et al.* (1985). The PCR product of 3.3 kbp was purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL41. Plasmid pPFL41 was identified by restriction patten and sequence analysis.

(Paragraph at page 58, line 18 through page 59, line 5.) The approximately 1.47 kbp DNA fragment of the eryAI gene of *S. erythraea* was amplified by PCR using as primers the synthetic oligonucleotides: 5' - TACCTAGGCCGGGCCGGACTGGTCGACCTGCCGGGTT- 3' (SEQ ID No. 38) and 5' -ATGTTAACCGGTCGCGCAGGCTCTCCGTCT- 3' (SEQ ID No. 39) and plasmid pNTEP2 (Oliynyk, M. *et al.*, Chemistry and Biology (1996) 3:833-839; W098/01546) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired

plasmid pJLK02 was identified by its restriction pattern and DNA sequencing.

(Paragraph at page 59, lines 8-20.) The approximately 1.12 kbp DNA fragment of the *eryA*I gene of *S. erythraea* was amplified by PCR using as primers the synthetic oligonucleotides: 5' -ATGTTAACGGGTCTGCCGCGTGCCGAGCGGAC- 3' (SEQ ID No. 40) and 5' -CTTCTAGACTATGAATTCCCTCCGCCCAGC- 3' (SEQ ID No. 41) and plasmid pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid, pJLK03 was identified by its restriction pattern and DNA sequencing.

(Paragraph at page 63, lines 12-26.) The approximately 2.2 kbp DNA segment of the rapB gene of *S. hygroscopicus* encoding the reductive loop of module 10 was amplified by PCR using as primers the synthetic oligonucleotides: 5' -TAAGATCTTCCGACGTACGCGTTCCAGC- 3' (SEQ ID No. 42) and 5' -ATGCTAGCCACTGCGCCGACGAATCACCGGTGG- 3' (SEQ ID No. 43) and as template an approximately 7 kbp fragment, which has been obtained by digestion of cosmid cos 26 (Schwecke, T. *et al.* (1995) Proc. Natl. Acad. Sci. USA 92:7839-7843) with *Sca*I and *Sph*I. The PCR product was treated with T4 polynucleotide kinase and then

ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content.

(Paragraph at page 64, line 17 through page 65, line 5.) The approximately 6.1 kbp DNA segment of the erythromycin PKS gene cluster of S. erythraea encoding the DNA fragment from the beginning of the ACP of module 2 to the beginning of the ACP of module 3 was amplified by PCR using as primers the synthetic oligonucleotides: 5' -TACCTGAGGGACCGGCTAGCGGGTCTGCCGCGTG- 3' (SEQ ID No. 44) and 5' -ATGCTAGCCGTTGTGCCGGCTCGCCGGTCGGTCC- 3' (SEQ ID No. 45) and plasmid pBAM25 (published pBK25 by Best, D J et al. Eur J Biochem (1992) 204: 39-49) as template. The PCR product was treated with T4 polynuclectide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK50 was identified by its restriction pattern and DNA sequencing.

(Paragraph at page 70, line 11 through page 71, line 3.) The approximately 1.8 kbp DNA segment of the monensin PKS gene cluster of Streptomyces cinnamonensis encoding part of the

ACP of the loading module 1 and KS of module 1 was amplified by PCR using as primers the synthetic oligonucleotides: 5' - CGTTCCTGAGGTCGCTGGCCCAGGCGTA- 3' (SEQ ID No. 46) and 5' - CGAAGCTTGACACCGCGGCGCGGCGCGG- 5' (SEQ ID No. 47) and a cosmid containing the 5' end of the monensin PKS genes from *S. cinnamonensis* or alternatively chromosomal DNA of *S. cinnamonensis* as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pPFL45 was identified by its restriction pattern.

(Paragraph at page 71, line 21 through page 72, line 2.) For the PCR amplification for plasmid pMO09, the following synthetic oligonucleotides were used as mutagenic primers, one containing a MunI site and the other a PstI site: 5' - GCGCGCCAATTGCGTGCACATCTCGAT- 3' (SEQ ID No. 48) and 5' - CCTGCAGGCCATCGCGACGACCGCGACCGGTTCGCCG- 3' (SEQ ID No. 49).

(Paragraph at page 72, lines 3-7.) For the PCR amplification for plasmid pM010, the following synthetic oligonucleotides were used as mutagenic primers, one containing a HindIII site and the other an EcoRV site: 5' -

GTCTCAAGCTTCGGCATCAGCGGCACCAA- 3' (SEQ ID No. 50) and 5' - CGTGCGATATCCCTGCTCGGCGAGCGCA- 3' (SEQ ID No. 51).

(Paragraph at page 72, line 9 through page 73, line 3.) For the PCR amplification for plasmid pMO13, the following synthetic oligonucleotides were used as mutagenic primers, one containing a PstI site and the other a HindIII site: 5' - GATGGCCTGCAGGCTGCCCGGCGGTGTGAGCA- 3' (SEQ ID No. 52) and 5' - GCCGAAGCTTGAGACCCCCGCCCGGCGCGGTCGC- 3' (SEQ ID No. 53) PCR was carried out on pNTEP2 (GB97/01810) as template using Pwo DNA polymerase and one cycle of: 96°C (1min); annealing at 50°C (3min); and extension at 72°C (1min), and 25 cycles of: 96°C (1min); annealing at 50°C (1min); and extension at 72°C (1min) in the presence of 10% (vol/vol) dimethylsulphoxide. The products were end-repaired and cloned into pUC18 digested with *Sma*I and the ligation mixture was transformed into *E.coli* DH 10B. Plasmid DNA was prepared from individual colonies. The desired plasmids for pMO09 (3.8kbp), pMO10 (3.9 kbp) and pMO13 (4.3 kpb) were identified by their restriction pattern and DNA sequencing.

Marked-Up Version of Amended Claims

16. (Amended) A [PKS] polyketide synthase (PKS) multienzyme for use in producing a [target] polyketide having substantially exclusively a desired starter unit, said PKS multienzyme comprising a loading module and a plurality of extension modules, wherein said loading module is adapted to load an optionally substituted malonyl and then to effect decarboxylation of the loaded residue to provide a corresponding optionally substituted acetyl residue for transfer to an adjacent one of said extension modules, and wherein at least one of the extension modules is not naturally associated with a loading module that effects decarboxylation; with the proviso that the [target] polyketide produced by the polyketide synthase is not a 14-membered macrolide having a 13-methyl group due to incorporation of an [(unsubstituted)] unsubstituted acetate starter; said multienzyme having the ability to synthesize said [target] polyketide produced by the polyketide synthase.

17. (Amended) A type I polyketide synthase which comprises a loading module and a plurality of extension modules, wherein said loading module is adapted to load an optionally substituted malonyl and then to effect decarboxylation of the loaded residue to provide a corresponding optionally substituted acetyl residue for transfer to the first of said extension modules, and

wherein at least said first extension module is not naturally associated with a loading module that effects decarboxylation; with the proviso that (a) the synthase is not composed of the loading module of the tylosin polyketide synthase coupled to the spiramycin polyketide synthase minus its natural loading module; and (b) the [target] polyketide produced by the polyketide synthase is not a 14-membered macrolide having a [13methyl] 13-methyl group due to incorporation of an [(unsubstituted)] unsubstituted acetate starter unit.

18. (Amended) A synthase according to claim [1] 17, wherein the decarboxylating functionality of the loading module is provided by a [ketosynthase-type] ketosynthase (KS)-type domain which differs from a KS domain of an extension module by having a glutamine residue in place of cysteine in the active site.

19. (Amended) A synthase according to claim [1] 17, wherein the decarboxylating functionality of the loading module is provided by a [CLF-type domain] polypeptide of the type which is alternatively designated as chain length factor (CLF) or ketosynthase (KS) β domain.

20. (Amended) A synthase according to claim [1] 17, wherein the loading module's loading functionality is provided by an

acyltransferase-type domain having an arginine residue in the active site.

22. (Amended) A type I polyketide synthase according to claim [1] 17, wherein the loading and decarboxylating functionality is provided by a KSq-Atq pair derived from a ketosynthase (KS)-acyltransferase (AT) [KS-AT] pair of domains which naturally occur together in an extension module wherein KSq represents the N-terminal ketosynthase-like domain of a loading module in which there is a glutamine residue in place of the active site cysteine residue of a KS domain of an extension module which is essential for beta-ketoacyl-ACP synthase activity and wherein ATq represents an AT domain as found immediately C-terminal of a KSq domain.

26. (Amended) A type I polyketide synthase according to claim 20, wherein said acyltransferase domain [is specific for loading with hydroxymethylmalonyl] corresponds to the acyltransferase of module 6 of the neddamycin polyketide synthase.

28. (Amended) A synthase according to claim [1] 17, wherein the loading module includes an acyl carrier protein.

29. (Amended) A synthase according to claim [1] 17, wherein at least the KSq domain of said loading module corresponds to the KSq domain of the loading module of the PKS multienzyme of oleandomycin, spiramycin, niddamycin, methymycin, tylosin or monensin wherein KSq represents the N-terminal ketosynthase-like domain of a loading module in which there is a glutamine residue in place of the active site cysteine residue of a KS domain of an extension module which is essential for beta-ketoacyl-ACP synthase activity.

30. (Amended) A type I polyketide synthase according to claim [1] 17, wherein said polyketide synthase is adapted to synthesize a polyketide selected from

(a) 12- and 16-membered macrolides with acetate starter units;

(b) 12, 14 and 16-membered macrolides with propionate starter units;

(c) variants of rifamycin, avermectin, rapamycin, immunomycin and FK506 with acetate starter units or propionate starter units; or

(d) a polyketide wherein the starter unit gave rise to a sidechain selected from allyl and hydroxymethyl.

38. (Amended) A type I polyketide synthase which comprises a loading module and a plurality of extension modules, wherein said loading module is adapted to load an

optionally substituted malonyl and then to effect decarboxylation of the loaded residue to provide a corresponding optionally substituted acetyl residue for transfer to the first extension module, and wherein at least part of the first extension module is heterologous to said loading module or at least a domain thereof; with the proviso that (a) the synthase is not composed of the loading module of the tylosin [PKS] polyketide synthase coupled to the spiramycin [PKS] polyketide synthase minus its natural loading module; and (b) the synthase is not adapted to direct the synthesis of a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter unit.

39. (Amended) A type I polyketide synthase enzyme or part thereof comprising a loading module of the form:
(decarbox) (AT) (ACP) where (ACP) represents an acyl carrier protein
(AT) represents an acyltransferase domain operative to load selectively a substrate selected from optionally substituted malonate units onto the ACP, and
(decarbox) represents a domain operative to decarboxylate an optionally substituted malonate substrate carried by the ACP, the (decarbox) being selected from a KSq domain and [CLF-type domains] a polypeptide of the type which is

alternatively designated as chain length factor (CLF) or ketosynthase (KS) β domain;

wherein the loading module includes domains or portions thereof derived from different sources and/or comprises engineered domains wherein KSq represents the N-terminal ketosynthase-like domain of a loading module in which there is a glutamine residue in place of the active site cysteine residue of a KS domain of an extension module which is essential for beta-ketoacyl-ACP synthase activity.

Abstract of the Disclosure

A polyketide synthase ("PKS") of Type I is a complex multienzyme including a loading domain linked to a multiplicity of extension domains. The first extension module receives an acyl starter unit from the loading domain and each extension module adds a further ketide unit which may under go processing (e.g. reduction). We have found that KSq domain possessed by some PKS's has decarboxy-lating activity, e.g. generating (substituted) acyl from (substituted) malonyl. The chain length factor domain (CLF; also designated the ketosynthase (KS) β domain) of type II PKS's has similar activity. By inserting loading modules including such domains into PKS's not normally possessing them it is possible to control the starter units used.